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EXAMINER	
GOLDBERG, JEANINE ANNE	
ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 03/22/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/872,493	KENNY ET AL.	
	Examiner	Art Unit	
	Jeanine A Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 December 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 3-35* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 3-35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. This action is in response to the papers filed December 30, 2003. Currently, claims 1, 3-35 are pending. All arguments have been thoroughly reviewed but are deemed non-persuasive for the reasons which follow.

2. Any objections and rejections not reiterated below are hereby withdrawn.

3. This action contains new grounds of rejection.

Priority

4. This application claims priority to provisional application 60/209,139, filed June 2, 2000.

Drawings

5. The drawings are acceptable.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

6. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was

not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

7. Claims 1, 3-4, 6-23, 27-33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Antao et al. (Techniques in Quantification and localization of gene expression, page 81-93, June 1999) in view of Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

Antao et al. (herein referred to as Antao) teaches a method for in situ detection of a nucleic acid analyte within a sample based on bDNA hybridization by a) preparing the sample by immobilizing, permeabilizing using proteinase K b) contacting with a probe wherein at least a portion of the target probe is complementary to at least a portion of the nucleic acid analyte c) washing with a detergent at a temperature of approximately 21 to 60 C (i.e. room temperature) and d) detecting the analyte-target probe complex on the substrate (Figure 6.2, page 84)(limitations of Claim 1). Specifically, Antao teaches fixing cells to a slide (page 84-95)(limitations of Claim 20). Antao teaches using a number of cell lines and cell strains including lymphoid cell line, blood cells (limitations of Claims 21-22, 32-33). The microscope slides are incubated in proteinase K (0.5 ug/ml in PBS)(page 85)(limitations of Claim 4). The slides are then contacted with target probes (page 86). The slides are rinsed in wash buffer at room temperature, i.e. between 21 and 60 degrees Celsius (page 86)(limitations of 1c). The hybridized probe is then detected using label probe with a fluorophore directly attached to it or is conjugated to an AP or horseradish peroxidase molecule (page 86)(limitations of Claims

16-17). Antao further teaches incubating the slides with amplifier. As seen in Figure 6.2, the preamplifier is complementary to a portion of the target probe and forms an analyte-target probe-preamplifier probe complex. Also, an amplifier is hybridized which is complementary to the preamplifier. Moreover, a Ap, fluorescent or chromagenic label is added to the amplifier probe and the presence of the label is detected. Antao teaches detecting two targets, HIV-1 viral RNA and hn RNP A2/B1 mRNA (limitation of Claim 3). The method of bDNA technology for *in situ* detection which allows for quantification and is specific, reproducible, easy to use and yields results within one day (page 83). Antao teaches using 50ul of preamplifier and amplifier at 0.4 fmol/ul, namely using 20 fmol of preamplifier and amplifier (limitations of Claims 14-15). Antao teaches using 50ul of 2-6 fmol/ul of target probe, namely 0.1-0.3 pmol of target (limitations of Claim 6). The washing solution is comprised of Tris, MgCl2, detergent and ZnCl2 (limitations of Claims 9-10). Antao teaches washing the slide at least twice (page 86)(limitations of Claims 11-12). Each of the wash steps are carried out a room temperature (limitations of Claim 13). Antao teaches centrifuging at 1,500 rpm for 6 minutes (page 85)(limitations of Claim 23).

Antao does not specifically teach washing the biological material with a detergent.

However, Xu teaches *in situ* hybridization of mRNA with hapten labeled probes (page 87). Haptenized nucleotides include biotin, digoxigenin, biotin. Xu also provides conditions for hybridization, washing, and detection of probe (page 95). The washing steps are performed at 55-65 degrees Celcius. Xu teaches that "for many probes, 55 degrees Celsius is best since stronger signals are obtained (page 95). Xu teaches that

0.1-0.5 ug of probe is used for hybridization. Xu teaches several washing solutions which use Triton, NaCl and KCl (limitations of Claim 7-10). Xu teaches that in some protocols, a higher concentration of detergent is used (1% Triton X-100) to give a cleaner background but can substantially reduce the signal (page 96).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the known bDNA hybridization assay of Antao which does not specifically provide for a detergent in the wash with the wash solution of Xu which comprises Triton. The ordinary artisan would have been motivated to have used the detergent for the expected benefit taught in the art by Xu, so as to reduce the background. Although Xu also teaches that the signal may be reduced, the method of Antao is specifically designed for signal amplification, therefore, the ordinary artisan would not anticipate this would cause any negative effects on the detection. The ordinary artisan would have a reasonable expectation of success for using a detergent in the in situ method of Antao to reduce background signal and ensure the signal being amplified by Antao is in fact the signal desired and not background signal. Therefore, the ordinary artisan would have used a wash solution with detergent for the expected benefits taught in the art.

8. Claims 1, 3-4, 6-13, 16-17, 20-27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A

practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

Schaeren-Wiemers et al. (herein referred to as Schaeren) teaches *in situ* hybridization using digoxigenin-labelled cRNA probes. Schaeren teaches *in situ* hybridization methods for tissue sections and cultured cells using digoxigenin-labelled cRNA probes (abstract). Schaeren teaches mounting on a polylysine coated slide (page 433)(limitations of Claim 4, 20). Schaeren teaches washing in 5xSSC and 0.2 X SSC (page 433, col. 2)(limitations of Claim 11). Schaeren teaches using tissue sections, namely brains and cell cultures, namely optic nerves (page 433, col. 2)(limitations of Claim 21-22, 24-26). Schaeren teaches culturing cells, aspirating off the medium, thereby having used a centrifuge (limitations of Claim 23).

Schaeren does not teach using bDNA hybridization detection means for an in situ hybridization assay. Moreover, Schaeren does not specifically teach using specific concentrations of proteinase K for permeabilizing or washing at temperatures approximately 21-60 degrees Celcius.

However, Cao teaches that development of a non-isotopic in situ hybridization technique for detection of nucleic acid in archived sputum specimens using branched DNA technology (in situ bDNA) is successful.

Further, Nolte teaches branched DNA signal amplification for direct quantitation of nucleic acid sequences in clinical specimens. Nolte emphasizes that the number of target molecules is not altered in the method of bDNA and the resulting signal is directly proportional to the concentration of the target nucleic acid (page 202). The signal of direct hybridization rather than the nucleic acid itself is amplified and thus is directly

proportional to the amount of target sequence present in the clinical sample (page 214). BDNA is a nonenzymatic process and is less prone to sample-to-sample variation than with enzymatically mediated target amplification systems. Figure 1 illustrates the bDNA signal amplification assay (limitations of Claims 2). Nolte describes comparisons which were performed between various assays. Nolte teaches that as study by Butterworth teaches the bDNA assay was linear over three orders of magnitude and was the most sensitive assay being approximately 10 times more sensitive than the other assays. Butterworth teaches that the amplifier oligonucleotides enhance the chemiluminescent signal by having multiple branches of the same sequence which bind to the alkaline phosphatase labeled probes which reacts with Dioxetane to produce a chemiluminescent signal which is detected by a luminometer and the concentration of HBV-DNA is determined (limitations of Claim 16, 17). Nolte teaches using bDNA to detect HCV, for example (page 206)(limitations of Claim 3). Nolte suggests the application for bDNA in *in situ* hybridization assays (page 231).

Moreover, Decimo et al. provides basic conditions for conducting *in situ* hybridization assays. Decimo teaches pre-hybridization treatments of slides with proteinase K, 1 ug/ml (page 192). The hybridization is performed followed by washing steps. The post-hybridization washes were performed at 55 C for about 45 minutes (limitations of Claim 1cm 13). Decimo teaches that approximately 5-10 ul of hybridization mixture is required per cm2 of tissue section. Decimo teaches several wash steps following hybridization (page 195)(limitations of Claim 11-12).

Xu teaches *in situ* hybridization of mRNA with hapten labeled probes (page 87). Haptenized nucleotides include biotin, digoxigenin, biotin. Xu also provides conditions

for hybridization, washing, and detection of probe (page 95). The washing steps are performed at 55-65 degrees Celcius. Xu teaches that "for many probes, 55 degrees Celsius is best since stronger signals are obtained (page 95). Xu teaches that 0.1-0.5 ug of probe is used for hybridization. Xu teaches several washing solutions which use Triton, NaCl and KCl (limitations of Claim 7-10). Xu teaches that in some protocols, a higher concentration of detergent is used (1% Triton X-100) to give a cleaner background but can substantially reduce the signal (page 96).

With respect to Claim 6, Schaeren does not specifically teach using approximately 0.1 pmoles to 10 pmoles of the target probe. Schaeren teaches preparing hybridization mixture by adding 200 ng cRNA per ml hybridization buffer. Moreover, 200 ul hybridization buffer was used. However, Xu teaches average *in situ* probes are between 32-36 nucleotides in length. Thus, using a 34 nucleotide probe as an average length, 130 as the average molecular weight of each base, the probe is approximately 4,420 g/mol. Thus, there is approximately .91 pmoles of target probe used. Thus, performing the *in situ* hybridization with various length probes, probes of various sequences, would have been within the optimization of the ordinary artisan. Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

Claim 27 requires that the position of a nucleic acid analyte within a sample is determined as indicative of the position of the nucleic acid analyte in the cell. Thus, the mere detection of the nucleic acid within the cell would be indicative of the position of the nucleic acid within the cell. Thus, based upon the language of the claim, the claim only requires identifying the complex within a cell.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the *in situ* hybridization assay using digozxigenin-labelled cRNA probes with the improved bDNA hybridization method. Aside from the prior art teaches that branced DNA technology is used in situ for detecting mRNA, the skilled artisan would have recognized that a comparison of the ELISA-based cheiluminescent method of Schaeren could be modified and improved by substituting the ELISA-based chemiluminescent method with bDNA methods, as taught by Nolte to be more sensitive, precise and linear. Given the comparison of solution hybridization methods taught by Nolte, there would have been a reasonable expectation that the bDNA method would have been more sensitive, precise and linear *in situ*. Hybridization mechanisms *in situ* and in solution are analogous, thus, the ordinary artisan would have expected bDNA to act superiorly to ELISA-based chemiluminescent methods *in situ*, as exemplified by the in solution comparison. *In situ* hybridization detection of probes would be enhanced by signal amplification such as bDNA. The signal amplification would allow for greater luminescence and greater detection signals.

With respect to the specific conditions for performing *in situ* hybridization, the skilled artisan would have been motivated to have optimized the conditions. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the probe selection performed was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way

as compared to the closest prior art. Thus, performing the *in situ* hybridization with conditions provided by Decimo as optimal for ISH, would have been within the optimization of the ordinary artisan. Moreover, Xu provides specific motivation why to perform the method using a detergent in the wash step. Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

Response to Arguments

The response traverses the rejection. The response asserts Nolte provides no teachings whatsoever of specific hybridization steps that could be used for bDNA hybridization. This argument has been reviewed but is not convincing because the Figure of Nolte provides steps for performing bDNA method. The response asserts that no teachings whatsoever of the specific hybridization steps, however, it is unclear which steps the combination of references as a whole fail to teach. There is a reasonable expectation of success that the skilled artisan would be able to modify the teachings of Schaerens-Wiemers using the method of Nolte to obtain a bDNA hybridization assay. Thus for the reasons above and those already of record, the rejection is maintained.

9. Claim 5 is rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999) as

applied to Claims above, 1, 3-4, 6-13, 16-17, 20-27 and in further view of Sarto et al (US Pat. 6,022,689, February 2000).

Neither Schaeren, Cao, Nolte nor Decimo specifically teach permeabilizing with about 5-20 ug/ml of Proteinase K.

However, Sarto et al. (herein referred to as Sarto) teaches a method of in situ hybridization slide processes. As seen in Figure 2a, a slide is used, cells are placed on the top of the slide, labeled DNA probe is added to the slide, a coverslip is placed on the slide, and the slide is simultaneously denatured, hybridized followed by a wash step. Specifically, glass slides are used to immobilize cell samples by allowing the cell solution to dry (limitation of Claim 4)(col.6, lines 55-60). In the paraformaldehyde fixation method, the cells were treated with proteinase K at 2.5 ug/ml to 10 ug/ml (col. 8, lines 45-50)(limitation of Claim 5).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have used about 5-20 ug/ml of Proteinase K for permeabilizing cells prior to *in situ* hybridization. With respect to the specific conditions for performing *in situ* hybridization, the skilled artisan would have been motivated to have optimized the conditions. As noted in *In re Aller*, 105 USPQ 233 at 235, "More particularly, where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." Routine optimization is not considered inventive and no evidence has been presented that the probe selection performed was other than routine, that the products resulting from the optimization have any unexpected properties, or that the results should be considered unexpected in any way as compared to the closest prior art. Thus,

performing the *in situ* hybridization with conditions provided by Sarto as optimal for ISH, would have been within the optimization of the ordinary artisan. Therefore, using particular concentrations and temperatures would have constituted optimization on the part of the ordinary artisan.

Response to Arguments

The response traverses the rejection. The response asserts that the rejection did not make sense, as previously Xu was only used in a rejection of Claims 6-10. In view of the revised rejection of Claim 1, the rejection is appropriate. Thus for the reasons above and those already of record, the rejection is maintained.

10. Claims 14-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) and in further view of Kern et al. (J. Clin. Microbiol. Vol. 34, No. 12, pages 3196-3202, 1996).

Neither Schaeren, Cao, Nolte, Decimo, nor Xu specifically teaches using between 1 fmol and 10pmoles of preamplifier or amplifier.

Kern teaches a method of bDNA quantification of HIV. The method of kern teaches using .70 fmol of preamplifier per ul and 1.0 fmol of bDNA amplifier per ul. The 50ul of solution was used.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have used the specified conditions provided by Kern in the absence of specific conditions provided in Nolte for the amount of preamplifier and amplifier used in a bDNA assay. The ordinary artisan would have looked to the art to the ranges and units of preamplifier and amplifier used in bDNA assays to optimize the method of using bDNA and in situ hybridization. Thus, using approximately 1 fmol to about 10pmoles of amplifier and of preamplifier, would have been obvious to the ordinary artisan at the time the invention was made.

11. Claims 18-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Schaeren-Wiemers et al (Histochemistry, Vol. 100, pages 431-440, 1993) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical Approach, 1996, pages 183-198) or Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999) as applied to Claims 1-4, 6-13, 16-17, 20-27 above, and further in view of Siadat-Pajouh (J. of Hist. And Cytochemistry. Vol. 42, No. 11, pages 1503-1512, 1994).

Neither Schaeren, Cao, Nolte, Decimo, nor Xu specifically teach the method has a sensitivity to detect from 1-2 copies of the nucleic acid.

However, Siadat-Pajouh et al. (herein referred to as Siadat) teaches a method of detecting single-copy HPV genomic *in situ* (limitations of Claim 29). The method of Siadat is a highly sensitive and quantitative fluorescence-based *in situ* hybridization technique that can detect as few as one to five copies in the HPB cells using digoxygenin tail-labeled oligonucleotides (limitations of Claims 28, 30). The method is

quick and may be carried out in four and half hours (abstract). Siadat uses human cervical carcinoma cell lines (page 1504, col. 1)(limitations of claims 31-33) Siadat teaches that the commonly used non-isotopic ISH methods are based on labeling probes with biotin or digoxigenin. The signal can be amplified in various ways and detected either via an enzyme substrate or binding to a fluorochrome. Siadat teaches that the advantage of the enzyme substrate detection system is that the intensity of the signal increases as function of time, making it more sensitive compared with other systems (page 1503, col. 2). Siadat teaches that much of the optimization of *in situ* hybridization conditions is necessarily empirical in nature.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have performed the *in situ* hybridization method of Schaeren in view of Nolte with the optimized conditions of Decimo or Xu and further have optimized the *in situ* hybridization assay to detect a single copy of the nucleic acid analyte in a cell, as taught by Siadat. Siadat teaches the importance of detecting single copy nucleic acids and provides means by which the *in situ* hybridization may be performed. The ordinary artisan would have been motivated to have optimized the in situ hybridization assay to include detection of single copy targets.

12. Claims 28-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Siadat-Pajouh (J. of Hist. And Cytochemistry. Vol. 42, No. 11, pages 1503-1512, 1994) in view of Cao et al. (Abstract #2287, March 1998) and Nolte (Advances in Clinical Chemistry, Vol. 33, pages 201-235, 1998) and Decimo (Gene Probes 2: A practical

Approach, 1996, pages 183-198) and Xu (In Situ Hybridization, Chapter 4, pages 87-106, 1999).

Siadat-Pajouh et al. (herein referred to as Siadat) teaches a method of detecting single-copy HPV genomic *in situ* (limitations of Claim 29). The method of Siadat is a highly sensitive and quantitative fluorescence-based *in situ* hybridization technique that can detect as few as one to five copies in the HPB cells using digoxygenin tail-labeled oligonucleotides (limitations of Claims 28, 30). The method is quick and may be carried out in four and half hours (abstract). Siadat uses human cervical carcinoma cell lines (page 1504, col. 1)(limitations of claims 31-33). Siadat teaches that the method of FISH would be useful in detection and quantitation of a variety of viral genomes, oncogenes and drug resistant genes, in a variety of morphologically intact cells and tissues (limitations of Claims 31, 34). Siadat teaches that the commonly used non-isotopic ISH methods are based on labeling probes with biotin or digoxigenin. The signal can be amplified in various ways and detected either via an enzyme substrate or binding to a fluorochrome. Siadat teaches that the advantage of the enzyme substrate detection system is that the intensity of the signal increases as function of time, making it more sensitive compared with other systems (page 1503, col. 2). Siadat teaches that much of the optimization of *in situ* hybridization conditions is necessarily empirical in nature.

Siadat does not specifically teach using bDNA for signal amplification and detection of nucleic acid analytes *in situ.*

However, Cao teaches that development of a non-isotopic in situ hybridization technique for detection of nucleic acid in archived sputum specimens using branched DNA technology (in situ bDNA) is successful.

Moreover, Nolte teaches branched DNA signal amplification for direct quantitation of nucleic acid sequences in clinical specimens. Nolte emphasizes that the number of target molecules is not altered in the method of bDNA and the resulting signal is directly proportional to the concentration of the target nucleic acid (page 202). The signal of direct hybridization rather than the nucleic acid itself is amplified and thus is directly proportional to the amount of target sequence present in the clinical sample (page 214). BDNA is a nonenzymatic process and is less prone to sample-to-sample variation than with enzymatically mediated target amplification systems. Figure 1 illustrates the bDNA signal amplification assay (limitations of Claims 2). Nolte describes comparisons which were performed between various assays. Nolte teaches that as study by Butterworth teaches the bDNA assay was linear over three orders of magnitude and was the most sensitive assay being approximately 10 times more sensitive than the other assays. Butterworth teaches that the amplifier oligonucleotides enhance the chemiluminescent signal by having multiple branches of the same sequence which bind to the alkaline phosphatase labeled probes which reacts with Dioxetane to produce a chemiluminescent signal which is detected by a luminometer and the concentration of HBV-DNA is determined (limitations of Claim 16, 17). Nolte teaches using bDNA to detect HCV, for example (page 206)(limitations of Claim 3). Nolte suggests the application for bDNA in *in situ* hybridization assays (page 231).

Moreover, Decimo et al. provides basic conditions for conducting *in situ* hybridization assays. Decimo teaches pre-hybridization treatments of slides with proteinase K, 1 ug/ml (page 192). The hybridization is performed followed by washing steps. The post-hybridization washes were performed at 55 C for about 45 minutes

(limitations of Claim 1cm 13). Decimo teaches that approximately 5-10 ul of hybridization mixture is required per cm2 of tissue section. Decimo teaches several wash steps following hybridization (page 195)(limitations of Claim 11-12).

Xu teaches *in situ* hybridization of mRNA with hapten labeled probes (page 87). Haptenized nucleotides include biotin, digoxigenin, biotin. Xu also provides conditions for hybridization, washing, and detection of probe (page 95). The washing steps are performed at 55-65 degrees Celcius. Xu teaches that "for many probes, 55 degrees Celsius is best since stronger signals are obtained (page 95). Xu teaches that 0.1-0.5 ug of probe is used for hybridization. Xu teaches several washing solutions which use Triton, NaCl and KCl (limitations of Claim 7-10). Xu teaches that in some protocols, a higher concentration of detergent is used (1% Triton X-100) to give a cleaner background but can substantially reduce the signal (page 96).

With respect to Claim 35, depending upon the sample available the ordinary artisan would have been motivated to have used tissue samples as opposed to cells for analyzing the nucleic acids *in situ*. Therefore, using the methods taught in the art for preparation of tissue samples, would have been obvious to the skilled artisan at the time the invention was made. With specific respect to the tissue types recited in Claim 35, the skilled artisan would have been motivated to have used any of the tissue types provided depending upon the sample available for analyses. In situ hybridization has been used on biopsies from cancer patients, thus, any tissue biopsy which requires analyzation would be appropriate and the ordinary artisan would be motivated to analyze the biopsy tissue.

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the *in situ* hybridization assay using digozxigenin-labelled cRNA probes and signal amplification with the improved bDNA signal amplification hybridization method. The skilled artisan would have recognized that a comparison of the ELISA-based cheiluminescent method of Siadat could be modified and improved by substituting the ELISA-based chemiluminescent method with bDNA methods, as taught by Nolte to be more sensitive, precise and linear. Given the teachings of Siadat that signal amplifications may be performed in various ways, and comparison of several hybridization methods, there would have been a reasonable expectation that the bDNA method would have been more sensitive, precise and linear in situ. *In situ* hybridization detection of probes would be enhanced by signal amplification such as bDNA. The signal amplification would allow for greater luminescence and greater detection signals. Thus, the ordinary artisan would have been motivated to have used the signal amplification method of bDNA in the signal amplification method taught by Siadat because the skilled artisan would have recognized that the bDNA signal amplification method is a quantitative method which is very sensitive.

Response to Arguments

The response traverses the rejection. The response asserts suggesting that the teachings of Nolte lack procedural guidance. This argument has been reviewed but is not convincing because the response fails to provide any particular limitations of the claims in which Nolte lacks procedural guidance. Thus for the reasons above and those already of record, the rejection is maintained.

Conclusion

13. No claims allowable over the art.

14. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (571) 272-0743. The examiner can normally be reached Monday-Friday from 8:00 a.m. to 4:00 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (571) 272-0745.

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